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Identification of two new bacterial species, *Brevundimonas nasdae* and *Microbacterium trichothecenolyticum* from Kolavai lake, Chengalpattu, Tamil Nadu, India

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ABSTRACT

The present study reports the physiochemical and biological parameters of water and identification of organisms present in Kolavai Lake, Tamil Nadu, India. The lake is heavily polluted due to the dumping of municipal and agriculture wastes from surrounding localities. Water samples were collected from different places across the lake and examined. Eleven microorganisms were identified by primary and secondary biochemical assays and two bacterial samples were sequenced with 16S rRNA regimen to identify the species. Obtained results were compared with standard values. The new bacterial species identified belonged to taxa *Brevundimonas nasdae* and *Microbacterium trichothecenolyticum* which are uncommon in this area.

Keywords: Kolavai Lake, Pollution, 16S rRNA, *Brevundimonas nasdae, Microbacterium trichothecenolyticum.*

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INTRODUCTION

Kolavai Lake is the second largest lake in Kanchipuram District of Tamil Nadu, India, next to Madhuranthagam Lake. Kolavai Lake is located in Chengalpattu town and is well known for its perenniality. Water from this is supplied to Chennai city during summer months. The lake is now being polluted due to the rapid urbanization of Chengalpattu[1]. Kolavai Lake, spread across 802 hectares, has the capacity to hold nearly 476 million cubic feet of water. It is fed by nearly 25 tanks located upstream in places such as Paranur, Hanumanthapuram and Kunnavakkam, and has a catchment area spread over 53 sq. Km. As the lake is located in a rocky region, the ground water recharge is not much. This makes it essential to restore the lake to sustain water quality in the surrounding villages. At present, five million gallons of water are drawn from the lake daily and treated, to be used for Mahindra World City Industrial Park[2]. Although a rich water source, the water is not potable due to high pollution. It is high time to analyzes the various factors like, identifying the quality of water, types of pollutants present in it, types of microorganisms (both beneficial and harmful) present there in, effects of these microorganisms on abiotic and biotic components of the lake and surroundings etc. Water receives microorganisms from air, sewage, soil and other organic wastes. Faecal pollution of water leads to introduction of variety of intestinal pathogens that causes water borne diseases[3]. In developing countries like India, 80 percent infectious diseases are water borne and 50 percent of the deaths among the children are due to diarrhoeal diseases[4]. Sewage containing human excreta, however, is the most dangerous material that pollutes water. During the treatment of water, chlorination has been carried out to control bacterial population. However, as the water is supplied to distant parts of Chennai, it results in revival of bacterial population and sediment accumulation. It has been estimated that globally 1.20 billion people become stick annually due to poor quality of drinking water. Further, according to (WHO, 2006), 80% of diseases of human beings are caused through water and 1800 million man working days are lost in India due to such water born diseases[5, 6, 7]. Precautions/care should be taken to prevent contamination of drinking water from chlorine resistant parasites such as *Cryptosporidium* species and *Giardia*. Although, *E. coli* is the more precise indicator of faecal pollution, the count of thermo- tolerant coliform bacteria is an acceptable alternative. The present study aimed to isolate, identify the different types of organisms present in it and also to analysis of physical and chemical parameters of the lake water. The idea of this work was to suggest remedial measures to the authorities so that this lake could be used not only for drinking water but also for agriculture, fisheries and recreation.

MATERIALS AND METHOD

Sample Collection

Water samples were collected for examination from Kolavai Lake, Chengalpattu, in sterilized plastic bottles which were tightly closed. They were carefully transferred to the laboratory for analysis and stored at 4^{o}C aseptically before processing. In addition to water sample, fishes were collected to examine the presence of bacteria, present in fish-gills, fins, intestine and mouth.

Analysis of Physico-chemical parameters of Water Samples

Analysis for colour, odour, turbidity, total dissolved solids at 105^{0}C (mg/l), Calcium as (Ca) (mg/l) Magnesium as (Mg) (mg/l), total hardness as ($CaCO_3$) (mg/l), chlorides as (Cl) (mg/l), albuminoidal Nitrogen as (N) (mg/l), Nitrous Nitrogen as (N) (mg/l), Nitric Nitrogen as (NO_3) (mg/l), Oxygen absorbed (Tidy's 4 hours test) (mg/l), Hydrogen ion Concentration (pH), alkalinity to phenolphthalein as ($CaCO_3$) (mg/l), alkalinity to methyl orange ($CaCO_3$) (mg/l), Sulphates as (SO_4) (mg/l), Phosphates as (PO_4) (mg/l), Iron as (Fe) (mg/l), Fluorides as (F) (mg/l), Specific Conductance (micro Siemens/cm) were carried out by methods of (APHA, (1985), ASTM International (2003); Trivedy and Goel, (1986); Kodarkar, (1992)[8, 9, 10, 11]. All the different water samples collected (Sample A, B, C and D) from Kolavai Lake were compared with their corresponding values of these 20 parameters, comparing it with BIS permissible limit of potable water (BIS Indian Standards, 2009)[12]. The result of this water analysis was given by Chennai Metropolitan Water Supply and Sewerage Board, Quality Assurance Wing, Water Analyst's Laboratory, Chennai -600010.

Sterilization

All Glassware's and culture media used for experiment was sterilized with the help of autoclave at 121^{o}C, 15 lbs pressure for 15 min.

Serial dilution

Isolation of bacteria was performed by serial dilution plate technique using Differential Agar Media. Serial dilution in the range of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} was carried out.

Types of differential media used

17 different types of agar were used as a differential media for water sampling and 4 nutrient agar media were used for samples from fish organs. These media were Plate count agar, Glycerol Asparaginase agar, Glucose yeast peptone, Peptone yeast extract iron agar, Urea agar base, Potato dextrose agar, fish gills sample in nutrient agar, Brilliant green bile broth, Pikovskaya's agar, fish fins sample in nutrient agar, Simmon citrate agar, Yeast malt agar, Triple sugar iron agar, Oat

meal agar, Eosin Methylene blue agar, fish intestine sample in nutrient broth, Lactose broth agar, fish-mouth sample in nutrient agar, Skimmed milk agar, Starch casein agar and Motility yeast medium (Harold, 2002)[13]. All 17 different types of differential agar media in addition to nutrient agar were weighed for 100 ml and then autoclaved for 121°C, 15 lbs pressure to arrest the contamination prior to analyzing sample. All the autoclaved agar media were poured into petri plates to get solidified after getting warmed. The method adopted for this experiment was Quadrant streak plate method.

Streak plate method

To obtain well isolated discrete colonies the quadrant streak was used as described below. The inoculating loop was sterilized by heating and a loop full of inoculums was streaked on one quarter of a plate. The same process was done and streaking was done on the second, third and fourth quarters separately.

Sub culturing

In this experiment, solid to liquid and liquid to solid sub culturing has been performed to obtain pure culture. Sub culturing has been successful when the transferred organisms have grown in the new medium without contamination. In this experiment, solid to liquid and liquid to solid sub culturing has been performed to obtain pure culture. Differential media like Plate count agar, Urea agar base, Brilliant green bile agar, Simmon citrate agar, Triple sugar iron agar, Oat meal agar, Eosin Methylene blue agar, Skim milk agar and Starch casein agar were sub cultured by solid to solid sub culturing method.

For solid to liquid subculture

Glycerol Asparginase agar, Glycerol yeast peptone agar, Peptone yeast extract iron agar, Potato dextrose agar, Pikovskayas agar, Yeast malt agar, Lactose broth, Motility yeast medium and inoculum samples of Fish –Gills, Fins, Mouth and Intestine on Nutrient broth were sub cultured by solid to liquid sub culturing method (HSDU Biology and Biotechnology Microbiological Techniques Intermediate) (Harold, 2002; Zaved et al, 2008)[13, 14]. Sterilized glass slides were used or smearing the bacterial samples by standard methods, heat killed the sample with and then stained for microscopic observation.

Staining the smear

The sample was flooded with crystal violet and allowed to set for 60 seconds. The slide was rinsed in slow running water. Then the sample was flooded with Gram’s Iodine for 60 seconds and rinsed with water. The slide was decolorized with 95% ethyl alcohol for 30 seconds and was flooded with

Safranin for 60 seconds and rinsed with water. The slide was air dried for few seconds and observed under the microscope.

Biochemical test for Identification of Unknown Organisms

The following standard tests were performed for identifying the various microorganisms (Harold, 2002; Collins *et. al*, 1989)[13, 15].

1. Oxidase Test
2. Catalase Test
3. Methyl Red and Voges Proskauer Test
4. Citrate Utilization Test
5. Indole Test
6. Triple Sugar Iron Test

RESULTS AND DISCUSSIONS

Bacterial identification service by genotyping using 16S rRNA

i. Genomic DNA isolation and PCR analysis

Genomic DNA was extracted from overnight grown cultures of the selected bacterial isolates using QIAGEN DNA isolation kit (Qiagen), suspended in 100 µl of elution buffer (10 mM/L Tris-HCl, pH 8.5) and quantified by measuring OD at 260 nm. PCR amplification was performed using a 50 µL reaction mixture containing 100 ng of template DNA, 20 µmol of 16S rRNA primers, 200 µM of dNTPs, 1.5 mM of $MgCl_2$, 1U of *Taq* DNA polymerase (MBI Fermentas) and 10 µL of 10x *Taq* polymerase buffer. The sequences of 16S rRNA primers used were as follows.

27f: (5'-AGAGTTTGATCCTGGCTCAG-3')

1522r: (5′-AAGGAGGTGATCCANCCRCA-3')

Amplification was carried out with an initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 56°C for 45 sec, extension at 72°C for 1 min and final extension at 72°C for 5 min using a thermocycler (iCycler; Bio-Rad Laboratories, CA). PCR products were analyzed on 1% agarose gel for 16S rRNA amplicons in 1x TBE buffer at 100 V. (Figure 1).

Table 1. Results showing the biochemical tests and their results of the various microorganisms in various media.

S. No	Types Of Agar Sample	Physical Appearance Of Colonies	Oxidase	Catalase	MR	VP	Citrate	Triple Sugar Iron	Indole
1	PCA	Fibrous white (milky)	+	+	-	-	+	No acid production i.e. Alkaline	_
2	Gly A.A	Transparent white color	+		-	+	+	Butt-yellow color, acid produced due to fermentation	_
3	PYEIA	Lightly whitish and brownish color	+	+	+	-	+	Butt –yellow color, acid produced ,and bottom black color, H_2S production.	_
4	UAB	Light yellow colour	+	+	-	+	+	No change	_
5	PDA	Milky white	+	-	-	-	+	No change	_
6	Fish Gills		+		-	-	+	Butt-yellow color, butt slightly raised –gas bubbles formation	_
7	B. Green	White color	+	-	+	-	+	Butt-yellow color, butt slightly raised –gas bubbles formation	_
8	PKVS	White color	+	-	-	-	-	Butt-yellow color, acid produced due to fermentation	_
9	Fish Fins		+	-	+	-	+	Butt-yellow color, acid produced due to fermentation	_
10	Si CA	Purple and red color colonies	+	-	+	_	+	Butt- pink color, No acid produced	_
11	YMA	Yellowish thin round fibres	+	+	+	-	+	No change	_
12	TSIA	Blackish due to H2S production	+	-	+	-	-	Slant –yellow color	_
13	OMA	Brown thin colonies	+	+	+	-	+	Butt-yellow color, acid produced due to fermentation	_
14	EMB	Light pinkish	+	-	-	-	+	Butt-yellow color, acid produced due to fermentation	_
15	Intestine		+		+	-	+	Butt-yellow color, acid produced due to fermentation	_
16	Lac B	Fibrous white	+	-	-	-	+	No change	_

17	Mouth		+		+	-	+	Butt and slant -yellow color, acid produced due to fermentation	_
18	PCA	Hazy white color	+	+	+	-	-	Butt -yellow color, acid produced due to fermentation, Butt slightly raised- gas production.	_
19	PCA	Milky white	+	+	-	-	+	Butt- yellow color, acid produced due to fermentation	_
20	Gly PA	White color	+	+	-	-	+	No change	_
21	SK MA	Light yellowish color colonies	+	-	-	-	+	No change	_
22	PK	Lime color colony	+	-	-	-	+	Butt-yellow color, and H_2S gas production	_
23	SCA	Light orange color	+		-	+	-	No change	_
24	UAB	Light grey color	+	-	-	-	+	Butt- yellow color, acid produced due to fermentation	_
25	PYEIA	Orange color colonies	+	+	-	-	+	Butt-yellow acid produced	_
26	MYM	Colorless	+	+	-	-	+	No acid produced	_
27	GYPA	White color	+	+	-	+	+	Butt yellow- acid is produced	_
28	Lac B	Fibrous white	+	-	-	-	+	Butt- slight yellow Moderate acid production .	_
29	SCA	White transparent	+		-	+	+	No change	_
30	PDA		+	-	-	-	+	Butt- yellow color, acid produced due to fermentation	_
31	PYEIA	Orangish color	+	+	-	+	+	Butt-moderately gas production.	_
32	UAB	Light grey color	+	+	-	-	+	Slant-black color, No change	_
33	MYM	White color	+	+	-	-	+	Slant –Black color	_
34	SKMA	Yellowish color colonies	+	-	-	-	+	No change	_
35	PKVS	White color	+	-	-	-	+	Butt-moderately yellow, so moderately acid production	_
36	Gly PA	White color	+	+	-	-	+	Butt- yellow color, acid is produced due to fermentation.	_

Table 2. **Showing the analysis report of water samples collected from Kolavai Lake, Chenaglapttu, Tamil Nadu, India.**

S.N	**Parameters**	**Sample Ranges**				**BIS : PERMISSIBLE LIMIT**
		Table 1	**Table 2**	**Table 3**	**Table 4**	
1	Colour	Yellowish	Yellowish	Yellowish	Yellowish	
2	Odour	Earthy	Earthy	Earthy	Earthy	
3	Turbidity (N.T.U)	24	20	22	20	10
4	Total dissolved solids ot 105^0 C(mg/l)	450	506	445	450	2000
5	Calcium (As Ca) (mg/l)	29	21	24	26	200
6	Magnesium (As mg) (mg/l)	27	36	29	28	
7	Total Hardness (As $CaCO_3$) (mg/ l)	184	200	180	180	600
8	Chlorides (As Cl) (mg/l)	104	116	104	108	1000
9	Ammoniacal Nitrogen (As N) (mg/l)	0.02	0.02	0.02	0.02	
10	Albuminoid Nitrogen (As N) (mg/l)	0.56	0.48	0.22	0.48	
11	Nitrous Nitrogen (As N) (mg/l)	Nil	Nil	Nil	Nil	
12	Nitric Nitrogen (As NO_3) (mg/l)	-	-	-	-	
13	Oxygen absorbed (Tidy's 4 hours test) (mg/l)	4.35	4.65	4.68	4.71	
14	Hydrogen Ion Concentration (pH)	8.3	8.2	8.4	8.2	6.5 to 8.5
15	Alkalinity to phenolphthalein (As $CaCO_3$) (mg/l)	Nil	Nil	Nil	Nil	
16	Alkalinity to Methyl orange (As $CaCO_3$) (mg/l)	156	172	156	156	600
17	Sulphates (As SO_4) (mg/l)	38	48	38	36	400
18	Phosphates (As PO_4) (mg/l)	0.010	0.010	0.010	0.010	
19	Iron (As Fe) (mg/l)	0.1	Trace	Trace	Trace	1.0
20	Fluorides (As F) (mg/l)	0.1	0.1	0.1	0.1	1.5
21	Specific Conductance (micro Siemens/cm)	690	780	685	695	

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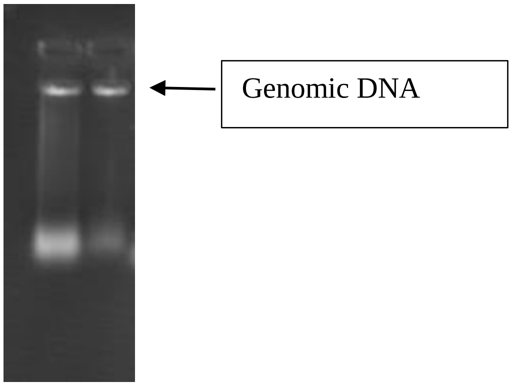

(LANE 1: RED COLONY; 2: YELLOW COLONY)

Figure1. Genomic DNA of selected two bacterial isolates

ii. Sequence analysis of PCR products

The 16S rRNA amplified fragments were purified using the QIA quick gel extraction kit (Qiagen, Valencia, CA) from the agarose gel and sequencing using automated DNA sequencer (Model 3100, Applied Biosystems, USA). The sequences were analysed using the option Basic Local Alignment Search Tool (BLAST) software available in NCBI [16]

Phylogenetic analysis

The sequences of these 16S rRNA genes were compared against the sequences available from GenBank using the BLASTN program (Altschul *et al*, 1997) and were aligned using CLUSTAL W software (Thompson *et al*, 1994)[17, 18]. Distances were calculated according to Kimura's two-parameter correction (Kimura, 1980)[19]. Phylogenetic trees were constructed using the neighbour-joining method (Saitou and Nei, 1987)[20]. Bootstrap analysis was done based on 1000 replications (Felsenstein, 1985)[21]. The MEGA4 package was used for all analyses as described by Tamura *et al*, 2007, (Figure 2, Figure 3, Figure 4, Figure 5 and Figure 6.

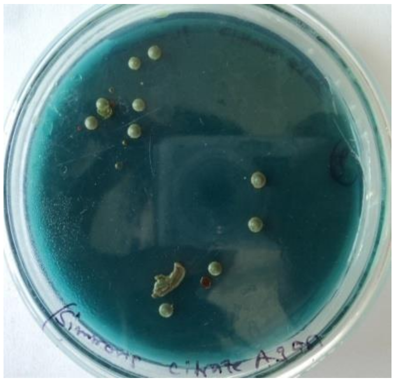

Figure 2. Simmon citrate agar Culture plate Showing Red Colony Red Colony_001(1402 bp)

Results of Genomic DNA of selected two bacterial isolates

Genomic DNA of selected two bacterial isolates Based on the 16S rRNA gene sequences BLAST analysis and Phylogeny analysis clearly revealed that the red colony is belong to the taxa *Brevundimonas nasdae* and yellow colony is belong to the taxa *Microbacterium trichothecenolyticum*. The study aimed at isolating and identifying the microorganisms present in Kolavai lake, in Chenalapattu, Tamil Nadu, India. This lake is being loaded with town and agricultural drains thus making it unfit for drinking. Similar studies were conducted on lake systems elsewhere also confirm the spoilage of drinking water due to agriculture and municipal wastes[23. 24]. Since Kolavai lake is a perennial lake we have suspected the presence of some bacteria which might have lived there for quite some time and it was quite possible that due to the long exposure to the polluted water they could either mutate into more adapted strains or might have changed given rise to new sub species or even new species!! We have primarily identified 11 genera of bacteria by usual bacteriological methods in the water namely, *Enterobacter, Proteus, Hafnia, Edwardsilla, Salmonella, Shigella, Klebsiella, Serratia, Morgenella, Providencia and Citrobacter*. During our study we have found some unsusal red colonies in Simmon citrate agar Culture plates and some yellow colonies in Starch casein agar plates. These colonies were of interest and they were subjected to 16S RNA probes to identify their phylogeny. The two organisms identified using 16s Ribosomal method as red colour colony *Brevundimonas nasdae* and yellow colour colony *Microbacterium trichothecenolyticum* from collected lake water sample. *Brevundimonas nasdae* was isolated from Russian space research Laboratory (Li *et al*, 2004)[26]. This genus was also reported in soil samples (Choi *et al*, 2010; Yoon et al, 2006; Kang *et al*, 2009)[27, 28, 29]. There is no report of this species from Indian water or soil. The turbidity value exceeded the permissible limit of drinking water standards and high value of oxygen absorbed indicates contamination. Hence the water is unfit for potable use according to Chennai metro water and sewerage board.

Figure 3. Simmon Sitrate Agar Culture plate Showing Red Colony

Sequences of the samples

CCGCGGTTTACGTTCAGTTTACCATGCGAGTCTGACGAACTGCTTCGGAGTTGCGTGG
CGGACGGGTGAGTAACACGTGGGAACGTGCCTTTAGGTTCGGAATAACTCAGGGAAA
CTTGTGCTAATACCGAATGTGCCCTTCGGGGGAAAGATTTATCGCCTTTAGAGCGGCC
CGCGTCTGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCAGTAGCT
GGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGG
AGGCAGCAGTGGGGAATCTTGCGCAATGGGCGAAAGCCTGACGCAGCCATGCCGCGT
GAATGATGAAGGTCTTAGGATTGTAAAATTCTTTCACCGGGGACGATAATGACGGTAC
CCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGC
TAGCGTTGCTCGGAATTACTGGGCGTAAAGGGAGCGTAGGCGGACATTTAAGTCAGG
GGTGAAATCCCGGGGCTCAACCTCGGAATTGCCTTTGATACTGGGTGTCTTGAGTATG
AGAGAGGTGTGTGGAACTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAAC
ACCAGTGGCGAAGGCGACACACTGGCTCATTACTGACGCTGAGGCTCGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATTGCTAGTT
GTCGGGATGCATGCATTTCGGTGACGCAGCTAACGCATTAAGCAATCCGCCTGGGGA
GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCACCTTTTGACATGCCTGGA
CCGCCACGGAGACGTGGCTTTCCCTTCGGGGACTAGGACACAGGTGCTGCATGGCTGT
CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCA
TTAGTTGCCATCATTTAGTTGGGAACTCTAATGGGACTGCCGGTGCTAAGCCGGAGGA
AGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACAGGGTGGGCTACACACGTGCTA
CAATGGCGACTACAGAGGGTTAATCCTTAAAAGTCGTCTCAGTTCGGATTGTCCTCTG
CAACTCGAGGGCATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTG
AATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTTCTACCC
GAAGGGGCTGCGCTGACCGCAAGGAGGCAGGCGACCACGGTAGGGTCAGCGAACCG
GGGTGGAAAAG

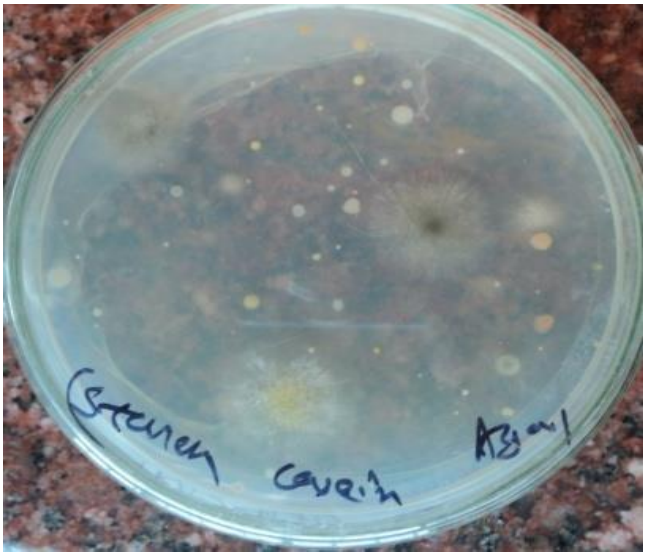

Figure 4. Starch casein agar Culture plate showing yellow colony

Sequences of the samples

Yellow Colony_001(1452 bp)

TAATGTTTCTAAGGCGAGTGAAAGCCAAGCTTGTTTTGTGGATCAGTGGCGAACGGGT
GACTAACTTGTGAGCAACCTGCCCTGGACTCTGGGATAAGCGCTGGAAACGGCGTCT
AATACTGGATATGAGCTCTCATCGCATGGTGGGGGTTGGAAAGATTTTTCGGTCTGGG
ATGGGCTCGCGGCCTGTCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGTCGACGG
GTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCC
TACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCAACG
CCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGGGAAGAAGCGA
GAGTGACGGTACCTGCAGAAAAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAA
TACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTT
GTCGCGTCTGCTGTGAAATCCCGAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCA
GACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGAT
ATCAGGAGGAACACCGATGGCGAAGGCATATCTCTGGGCCGTAACTGACGCTGAGGA
GCTAAAGGGTGGGGAGCAAACAGGCTTAGATACCCTGGTATTCCACCCCGTAAACGT
TGGGAACTAGTTGTGGGGACCATTCCACGGTTTCCGTGACGCAGCTAACGCATTAAGT
TCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGACCC
GCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGG
CTTGACATACACCAGAACACCGTAGAAATACGGGACTCTTTGGACACTGGTGAACAG
GTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCTATGTTGCCAGCACGTAATGGTGGGAACTCATGGGATACTGCC
GGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGTCTTG
GGCTTCACGCATGCTACAATGGCCGGTACAAAGGGCTGCAATACCGTGAGGTGGAGC
GAATCCCAAAAAGCCGGTCCCAGTTCGGATTGAGGTCTGCAACTCGACCTCATGAAGT
CGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGTCTTG
TACACACCGCCCGTCAAGTCATGAAAGTCGGTAACACCTGAAACAGGGGTGGCCCAA
CCAATGTGGAGGGAGCAAAACGAAGGTGGGATCGCTAATTTAAGGAAACAAAAATA

Phylogenetic tree analysis of the samples

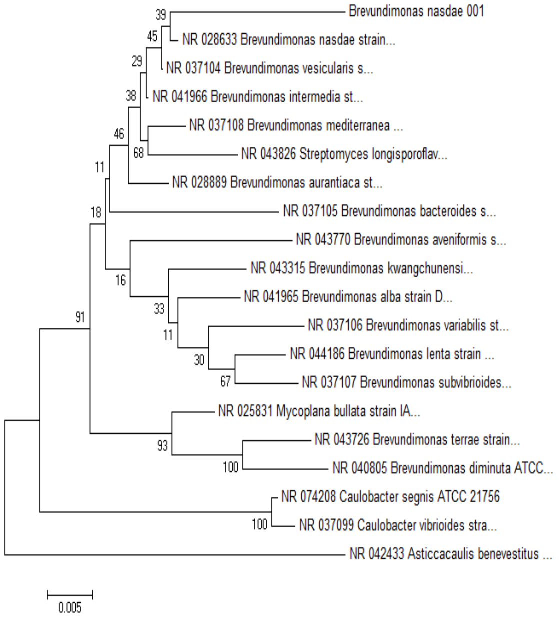

Figure 5. Phylogeny tree of Red colony

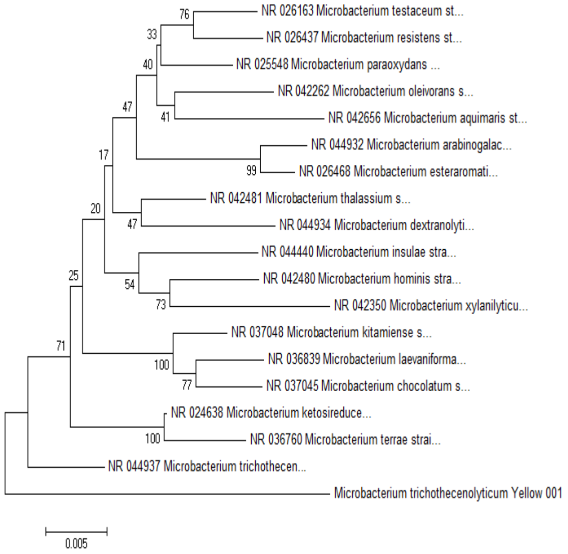

Figure 6. Phylogeny tree of Yellow colony

CONCLUSION

Futher work is on regarding the identification of other bacteria, their evolutionary and adaptive signficance with relation to the polluted water of Kolavai lake.

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ABBREVATIONS

UAB- UREA AGAR BASE

PYEIA- PEPTONE YEAST EXTRACT IRON AGAR

PDA- POTATO DEXTROSE AGAR

B.GREEN- BRILLIANT GREEN BRILE BROTH

PKVS- PIKOVSKAYA’S AGAR

SI C A- SIMMON CITRATE AGAR

YMA- YEAST MALT AGAR

TSIA- TRIPLE SUGAR IRON AGAR

OMA- OAT MEAL AGAR

EMB- EOSIN METHYLENE BLUE

LAC B - LACTOSE BROTH

PCA- PLATE COUNT AGAR

GLY P A- GLYCEROL YEAST PEPTONE EXTRACT

SKMA- SKIMMED MILK AGAR

SCA- STARCH CASEIN AGAR

MYM- MOTILITY YEAST MEDIUM

GY P A – GLYCEROL YEAST PEPTONE AGAR